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Type 2B von Willebrand's Disease in Thirteen Individuals From Five Unrelated Australian Families: Phenotype and Genotype Correlations

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Type 2B von Willebrand's disease (VWD) is due to a qualitative defect in von Willebrand factor (VWF) in which there is an increased affinity for the platelet glycoprotein Ib–IX–V receptor complex. Spontaneous binding of type 2B VWF to platelets and subsequent clearance from the plasma is thought to account for the characteristic phenotype of type 2B VWD. These gain-of-function mutations are due to single amino substitutions that are clustered within the functionally important A1 domain of VWF. We describe 13 individuals from five unrelated families in Australia with type 2B VWD, report their phenotypic abnormalities, and delineate their causative mutations. We confirm that the mutation Arg543Trp is also particularly common among families with type 2B VWD in Australia. Am. J. Hematol. 63:197–199, 2000. © 2000 Wiley-Liss, Inc.

Key words: von Willebrand's disease; type 2B; mutation

INTRODUCTION

Type 2B von Willebrand's disease (VWD) is a qualitative disorder which is characterized by an autosomal dominant mode of inheritance and loss of the largest von Willebrand factor (VWF) multimers. The VWF from these patients displays an increased affinity for platelets and binds to the glycoprotein Ib–IX–V complex in the absence of modulators such as ristocetin and botrocetin [1]. Spontaneous binding of type 2B VWF to platelets and subsequent clearance from the plasma is thought to account for the characteristic phenotype of type 2B VWD [2]. It is believed that this gain-of-function phenotype is due to the inactivation of an inhibitory domain. That is, the type 2B mutations mark segments in the VWF A1 domain that repress a separate GPIb binding site. To date, there have been more than twenty different "gain of function" mutations described responsible for the type 2B VWD phenotype. The majority of these defects are due to single amino acid substitutions that localise to a region within the functionally important A1 domain [3]. We describe 13 individuals from five unrelated families in Australia with type 2B VWD, report their phenotypic abnormalities, and delineate their causative mutations.

METHODS

Haemostasis Studies

Ristocetin-induced platelet aggregation (RIPA) was evaluated on patient platelet-rich plasma (PRP) by measuring the extent of agglutination following titration of ristocetin. Ristocetin cofactor activity (VWF:RCo) was performed with paraformaldehyde-fixed donor platelets and diluted patient plasma. Aggregation was induced by the addition of 1 mg/ml ristocetin as previous described [4]. FVIII coagulant (FVIII:C) activity determinations were performed using factor VIII-deficient plasma according to standard methods [5]. VWF antigen (VWF:Ag) levels were measured by enzyme-linked immunosorbent assay as previously described [6,7]. The VWF collagen binding assay (VWF:CBA) has been described elsewhere [6,7]. High-resolution multimeric analysis of VWF was performed by SDS-agarose gel electrophoresis [2].

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TABLE I. Type 2B VWD Patient Phenotype and Genotype Data

Patient	FVIII:C (%) NR = 50–200[a]	VWF:Ag (%) NR = 40–200	VWF:RCo (%) NR = 45–200	VWF:CBA (%) NR = 50–400	Mutation
I.1	38	39	ND[b]	21	Arg543Trp
I.2	47	32	5	4	Arg543Trp
I.3	64	32	5	4	Arg543Trp
I.4	41	ND	10	ND	Arg543Trp
II.1	43	35	9	10	Arg543Trp
III.1	ND	53	32	42	Arg543Leu
III.2	63	43	17	12	Arg543Leu
III.3	36	46	15	18	Arg543Leu
III.4	23	32	13	15	Arg543Leu
IV.1	62	55	6	ND	Arg543Trp
IV.2	58	68	33	28	Arg543Trp
V.1	32	27	ND	4	Arg545Cys
V.2	36	34	5	5	Arg545Cys

[a]NR = normal range.
[b]ND = not determined.

Enzymatic Amplification of VWF Exon 28 and Sequencing

Amplification of the VWF gene was performed using primers which amplified a 936-bp product encoding amino acid residues 463–743 of mature VWF [8]. Single-stranded DNA suitable for sequencing was obtained by solid-phase purification following attachment of the biotinylated PCR product to magnetic Streptavidin-coated beads (Dynabeads M280 Streptavidin, Dynal, Melbourne, Australia) and sodium hydroxide denaturation, according to manufacturer's instructions.

RESULTS

Coagulation Studies

In the current study, 13 individuals from five unrelated families with putative type 2B VWD were extensively characterized at the genotypic and phenotypic levels. All patients were studied using a variety of haemostatic tests (summarized in Table I). VWF:Ag and FVIII:C levels were either mildly reduced or borderline-normal in each individual. In contrast, VWF:RCo and VWF:CBA levels were each significantly reduced compared with corresponding VWF:Ag levels.

In normal individuals ristocetin-induced platelet aggregation (RIPA) usually only occurs at concentrations of ristocetin of 1 mg/ml and above. In all of the above cases, RIPA occurred at concentrations of 0.5 mg/ml of ristocetin (not shown), while in one individual it occurred at 0.25 mg/ml of ristocetin. The RIPA results demonstrated increased platelet sensitivity to reduced levels of ristocetin, a finding consistent with type 2B VWD. In one individual from each of four families (patients I.4, II.1, III.4, and V.1) VWF multimeric analysis was performed and each displayed a reduction in high molecular weight forms of VWF compared to that of pooled normal plasma (not shown).

Genomic Analysis

Sequencing of the 936-base pair exon 28 fragment revealed that individuals from three families (I, II, and IV) demonstrated a C to T mutation at nucleotide 3916 of VWF, resulting in an Arg543Trp amino acid change (Table I). Two individuals from a fourth family (patients V.1 and V.2) carried an Arg545Cys substitution due to a C to T mutation at nucleotide 3922. Four affected individuals from family III exhibited the recently reported G to T mutation at nucleotide 3915 resulting in a novel Arg543Leu amino acid substitution [8].

DISCUSSION

Type 2B VWD is a rare variant of VWD in which patient VWF demonstrates unregulated binding to the platelet GPIb-IX-V complex. Affected individuals are capable of synthesizing the complete repertoire of VWF multimers, as shown by normal VWF multimeric patterns in VWF isolated from platelets [2] and endothelial cells [9]. However, in these patients, the stimulated release of VWF by infusion of DDAVP gives rise to only transient increases in the levels of VWF released from endogenous tissue pools [10]. The defect is therefore thought to be due to the rapid clearance of the endogenous VWF molecule by abnormal binding of VWF to the platelet GPIb–IX–V complex, followed by platelet aggregation and clearance [10]. This also appears to explain the mild thrombocytopenia present in individuals with 2B VWD.

Type 2B VWD is characterized by normal or slightly reduced VWF antigen levels, while VWF:RCo and VWF:CBA levels are more significantly reduced. The

VWF:CBA utilises the discriminatory ability of a collagen preparation to selectively recognise the high molecular weight multimers of VWF and so permits discrimination between type 2 (in which high molecular weight multimers are reduced) and other forms of VWD [4,6]. Each of the individuals described in the present study displayed a greater reduction in their VWF:RCo and VWF:CBA activities compared with their VWF:Ag levels, features consistent with 2B VWD [4]. Further evidence for type 2B VWD derives from the fact that increased platelet agglutination at low concentrations of ristocetin provides a fairly specific assay to identify such defects. Ristocetin causes dose-dependent platelet aggregation when added to platelet-rich plasma, and this aggregation requires the interaction of plasma VWF with platelet GPIb–IX–V (11). All of the individuals described in the present study displayed platelet aggregation at low concentrations of ristocetin, that is, at 0.5 mg/ml of ristocetin, and occasionally at 0.25 mg/ml.

We described five families in Australia who exhibit features consistent with type 2B VWD. In order to confirm the diagnosis in these families, the genetic mutation was delineated in 13 individuals who displayed a phenotype that was consistent with this diagnosis. Three families carried the same Arg, 43Trp mutation, which is one of the most common defects found in patients with type 2B VWD [3,12]. The other three major loci involved in 2B VWD are Arg545 to Cys or Pro, Val553 to Met, and Arg578 to Gln or Leu [3,12–14]. A fourth family in this study exhibited the Arg545Cys defect, while the four individuals from family III have recently been shown to carry a novel Arg543Leu mutation [8]. Notably, each of the three point mutations responsible for type 2B VWD in these families involves loss of a positively charged arginine residue. These mutations all reside within a highly basic segment of VWF adjacent to the Asp514-Glu542 sequence, the potential glycoprotein Ib binding site [15]. This loss of charge within the A1 domain is consistent with an electrostatic model of VWF A1 domain activation. That is, it is believed that these VWF type 2B mutations interfere with the conformation of the A1 domain, allowing it to be constitutively turned on in the absence of activation and to facilitate GPIb binding [15]. Moreover, these gain-of-function type 2B mutations have recently been shown by X-ray crystallography to cluster to a corner of the VWF A1 domain [16,17]. This study of a number of unrelated individuals in Australia with type 2B VWD provides further insight into the relationship between phenotype and genotype.

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